

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representation of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

## **IMAGES ARE BEST AVAILABLE COPY**

**As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.**

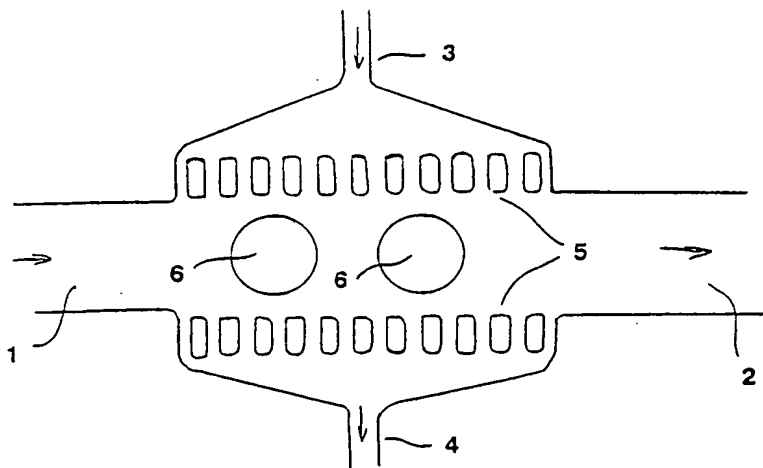
**THIS PAGE BLANK (USP10)**



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup> :</b> <b>G01N 33/543, 35/00 // G01N 21/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 94/11735</b> <b>(43) International Publication Date:</b> 26 May 1994 (26.05.94)
<b>(21) International Application Number:</b> PCT/FI93/00442 <b>(22) International Filing Date:</b> 28 October 1993 (28.10.93)  <b>(30) Priority data:</b> 925064                      9 November 1992 (09.11.92)    FI  <b>(71)(72) Applicant and Inventor:</b> SOINI, Erkki [FI/FI]; Krypingtonie 20, FIN-21610 Kirjala (FI).  <b>(74) Agent:</b> TURUN PATENTTITOIMISTO OY; P.O. Box 99, FIN-20521 Turku (FI).  <b>(81) Designated States:</b> AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).		<b>Published</b> <i>With international search report.</i>

**(54) Title:** METHOD AND APPARATUS FOR BIOAFFINITY ASSAYS



**(57) Abstract**

This invention relates to an automated multiparameter bioaffinity assay method. The method uses a mixture of prefabricated microparticles distributed in different categories and representing different analytes. The microparticles representing different categories contain different amounts of a fluorescent dye and are coated with biospecific reactants binding different analytes. The sample to be analysed is added and a mixture of biospecific secondary reactants labelled with a molecule generating or catalyzing luminescence or with a fluorescent or phosphorescent molecule is added to the said mixture. The fluorescent dye is activated, and the category of the microparticle is identified by means of the strength of the fluorescent emission. The biospecific binding reaction is measured by means of the signal from the molecule generating or catalysing luminescence or the signal from the fluorescent or phosphorescent molecule. According to the invention, the identification of the microparticle category and the measurement of the biospecific binding reaction, and possibly also dispensing, mixing, incubation and separation of the free fraction are carried out within a system of microchannels.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

Silicon technology allows to integrate micropump membranes, heating elements, temperature exchangers, temperature sensors, pressure sensors, optical sensors, other optical elements and electronics in the same silicon  
5 wafer with the microfluidistics.

The principal characteristics of the microfluidistics described above are the following:

- the fluid handling spaces are of such geometrical design that the microparticles can be moved at all times by a  
10 hydraulic mechanism, and the system does not contain pockets the size of or larger than the particle dimensions,
- the construction of the system is hermetic and sterile, the inputs and the outputs being subjected to biosafety control,
- 15 - the volume of liquid waste, sterilizable internally, is less than 10 - 100 microliter per analysis,
- the fluid is not in contact with any separate moving, wear-sensitive components,
- the dispensing system of the fluids is completely  
20 hydraulic (no liquid-gas interphase),
- the fluids are dispensed within a volume range of 1 nanoliter - 100 microliter),
- the accuracy of the handling of fluids is 0.1 % in the microlitre range and 1 - 5 % in the nanolitre range,
- 25 - the decontamination capacity of the liquid handling system (cross-contamination) is 0.0001 %,
- hydraulic mixing is provided for the reactants during dispensing and incubation, and the fluids to be mixed are not in contact with moving, wear-sensitive parts.

30 The complete automation described here comprises an apparatus into which fluid samples, e.g., blood serum, are fed through a thin tube. The necessary reactants, e.g., the suspension of microparticles and the necessary buffers and wash liquids are contained in purposeful and sterile (if  
35 needed) containers hermetically connected to the apparatus.

Samples and reactants are handled in the said system in a closed space and in microvolumes. The essential reactants of the said system are the following:

1. The microparticle suspension is a mixture of  
5 microparticles coated with different bioaffinity reactants, the diameter of the said particles being 1 - 200 micrometer. An example of commercial microparticles is the selection of Duke Scientific Inc. (USA). Magnetic microparticles can also be used.
- 10 2. A buffer is a fluid used to optimize the chemical environment during incubation. A buffer may also be used to wash the system.
3. A measuring fluid is a fluid used to optimize the  
15 chemical environment during the measurement of the fluorescence of the lanthanide chelate or during the measurement of phosphorescence or luminescence. A measuring fluid may incorporate an enhancer of the fluorescence of the lanthanide chelate (e.g. cofluorescence) and a reagent to eliminate agglutination of the microparticles.
- 20 A functional block diagram of the system is shown in Figure 2, comprising the following parts:
  1. A multiplace incubator 10, into which the sample, microparticle suspension and buffer are fed. The multiplace incubator incorporates microchannels, ice valves and  
25 temperature regulators, and allows random input of samples and simultaneous incubation. In the incubator the sample is mixed with the microparticle suspension. The incubation time of each sample is precisely controlled, and the bioaffinity reaction is not necessarily brought to  
30 equilibrium. The number of microparticles being in the incubator at any given time does not have a significant effect on reaction kinetics and precision but the system allows a precise count of the microparticles to be taken,

should that prove useful for the sake of precision. Different samples may be incubated for different lengths of time.

Bound reactant is separated from free reactant by, e.g.,  
5 the well-known by-pass washing-filtering method. An alternative method is to fix the microparticles in the incubator by means of magnetic field and wash the free reactant away, at the same time replacing the free reactant with the measuring fluid. The closed flow-through  
10 centrifuge described in FI patent No. 72660 or the filter method described in FI patent No. 67490 may also be used for said separation.

2. A pump 20 for the transfer of fluids in the microchannels and of the microparticle suspension into the  
15 detector. Pumping continues until the desired number of microparticles corresponding to each category have been measured. The micropump ensures the transport of the suspension into the detectors at precisely controlled speed, and the microparticle may be moving or stationary  
20 while measurement is taking place. The microparticle suspension is at this stage so dilute that the average inter-particle distance in the flow capillary is sufficient to allow the optical resolution of the signals they emit. The micropump moves the microparticle suspension in the  
25 microphotometric measuring channel at a predetermined speed along the center axis of the channel, the microparticles being centered by laminar flow, and the location of the particle may be determined in the direction of the flow, if necessary, with the precision of micrometers, at the focal  
30 point of the photometric detector. The liquid flow centers the particle in the transverse direction of the flow with the same precision. If the specific mass of the particle differs from that of the measuring fluid, the particle will, after being localized, that is, after liquid flow has  
35 stopped, either settle or rise against the wall of the horizontal measuring channel in a fraction of a second. At

this point a photometric, fluorometric or luminometric measurement of the particle may be taken with the accuracy and sensitivity required for the particular application.

The micropump described above may be, e.g., a bellow pump  
5 controlled by a computer-controlled stepping motor and an eccentric drive, the volume of fluid dispensed by the pump having a nonlinear relation to the rotation angle of the stepping motor, which allows transport of widely differing fluid volumes at a predetermined speed and with constant  
10 precision.

3. The element 30 used for identifying the microparticles is a photometric or fluorometric device incorporating a light source and a photodetector for the measurement of the dye contained in the microparticle and possibly for the  
15 measurement of the diameter of the microparticle. The category of each microparticle is identified by the system according to the strength of the signal from the detector. The information obtained from the identification process and from the measurement is used as calculation parameters  
20 in the standardization program for the particular analyte in a computer which calculates the concentration of the analyte in the sample based on the signal strength obtained from the detector (40) for the label. The identifying element can also detect agglutinated microparticles, which  
25 are not measured but are diverted away.

4. The detector 40 for a label is either a time-resolved fluorometer or phosphorometer or a luminometer. The measuring cell may be a commercial flow-through cell measuring, e.g., 0.25 by 0.25 mm in cross section and with  
30 an effective volume of less than 20 nanoliter, in which volume the signal of just one microparticle is measured at a time. Alternatively the measuring chamber is integrated in same silicon substrate with other functional parts.

The system further incorporates for the control of the



functional elements for fluid handling and for the control of the electrical functions, other required functions such as conventional pumps, valves, fluid containers and temperature regulators as well as the required number of  
5 electronic control elements and a computer for system control and for the standardization and computation of results.

A specialist in the field appreciates that the different applications of the said invention may vary within the  
10 scope of the claims to be presented in the following section. It will be appreciated that the methods of the present invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent to the artisan that other  
15 embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

## CLAIMS

1. A method for automated multiparameter bioaffinity assay using prefabricated microparticles distributed into different categories and representing different analytes,
  - in which method the microparticles belonging to different
  - 5 categories contain different amounts of a fluorescent dye,
  - in which method the microparticles of different categories are coated with biospecific reactants binding different analytes,
  - in which method the microparticles of different
  - 10 categories are mixed in a suspension into which suspension the sample is added, into said suspension a mixture of biospecific secondary reactants labelled with a molecule generating or catalysing luminescence or a fluorescent or a phosphorescent molecule is added,
  - 15 - in which method the fluorescent dye is activated and the strength of the fluorescent emission is measured separately for each particle for the identification of the microparticle category,
  - and in which method the label of the biospecific
  - 20 secondary reactant is activated and the biospecific binding reaction is measured by means of the signal from the molecule generating or catalysing luminescence or from the fluorescent or phosphorescent molecule, characterized by the use of a system of microchannels for the identification
  - 25 of microparticle categories and the measurement of biospecific binding reactions and possibly also for dispensing, mixing, incubation or the separation of the free fraction.
2. The method according to claim 1, characterized by fluid
- 30 transport in thin-walled microchannels manufactured of inert metal by electroformation or by fluid transport in microchannels made into a substrate manufactured with lithography and etching.
3. The method according to claim 1, characterized by the

use of a closed design of the fluid handling system formed by microchannels.

4. The method according to claim 1, characterized by the use of ice valves for the control of the operation of the  
5 system of microchannels and/or dispensing of fluids.

5. The method according to claim 1, characterized by the use of hydraulic dispensing of fluids so that essentially no liquid-gas interface exists in the microchannels during  
10 dispensing.

6. The method according to claim 1, characterized by a control of the flow of the microparticles in the system of microchannels, at varying speed, determined by a micropump precisely controlled by a computer.

15 7. The method according to claim 6, characterized by identification and measurement of the movement of the microparticles by means of microphotometric detectors and feeding the resulting data into the computer controlling the pump.

20 8. The method according to claim 6, characterized by the use of a micropump being a nonlinear bellows pump with constant precision over a wide range of volumes.

9. The method according to any one of the claims 1 - 8, characterized by the use of a sample register formed by  
25 microchannels and valves, for simultaneous incubation of the samples.

10. The method according to any one of the claims 1 - 9, characterized by separation of free and bound labelled reactants by extensive dilution or by exchanging the fluid  
30 phase containing the microparticles.

11. The method according to claim 10, characterized by

optimization of the new fluid phase resulting from exchange or dilution, to provide maximal assay sensitivity of the label.

12. The method according to claim 10, characterized by  
5 separation of the free reactant from the microparticles by dilution or by-pass flow filtering in a microchannel containing a plane or a device to detain the microparticles.
13. The method according to claim 12, characterized by the  
10 use of a device being a closed flow-through centrifuge.
14. The method according to any one of the claims 1 - 13, characterized by mixing of samples and reactants during dispensing and incubation by means of turbulent flow generated by hydraulic pressure pulses in a microchannel  
15 containing, in addition to the aforementioned fluids, a gas phase for the elastic reception of the pressure pulses.
15. The method according to any one of the claims 1 - 14, characterized by decontamination of the system of microchannels after preceding sample by pumping a mixture  
20 of fluid and gas through the system of microchannels.

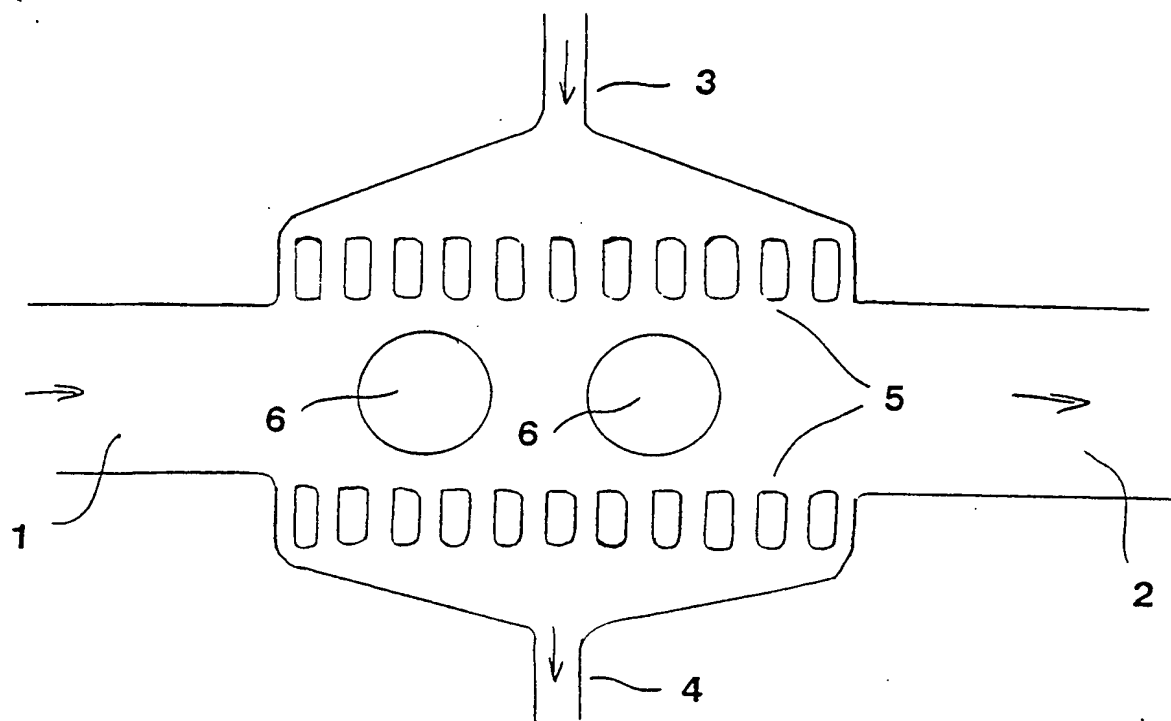


FIG. 1

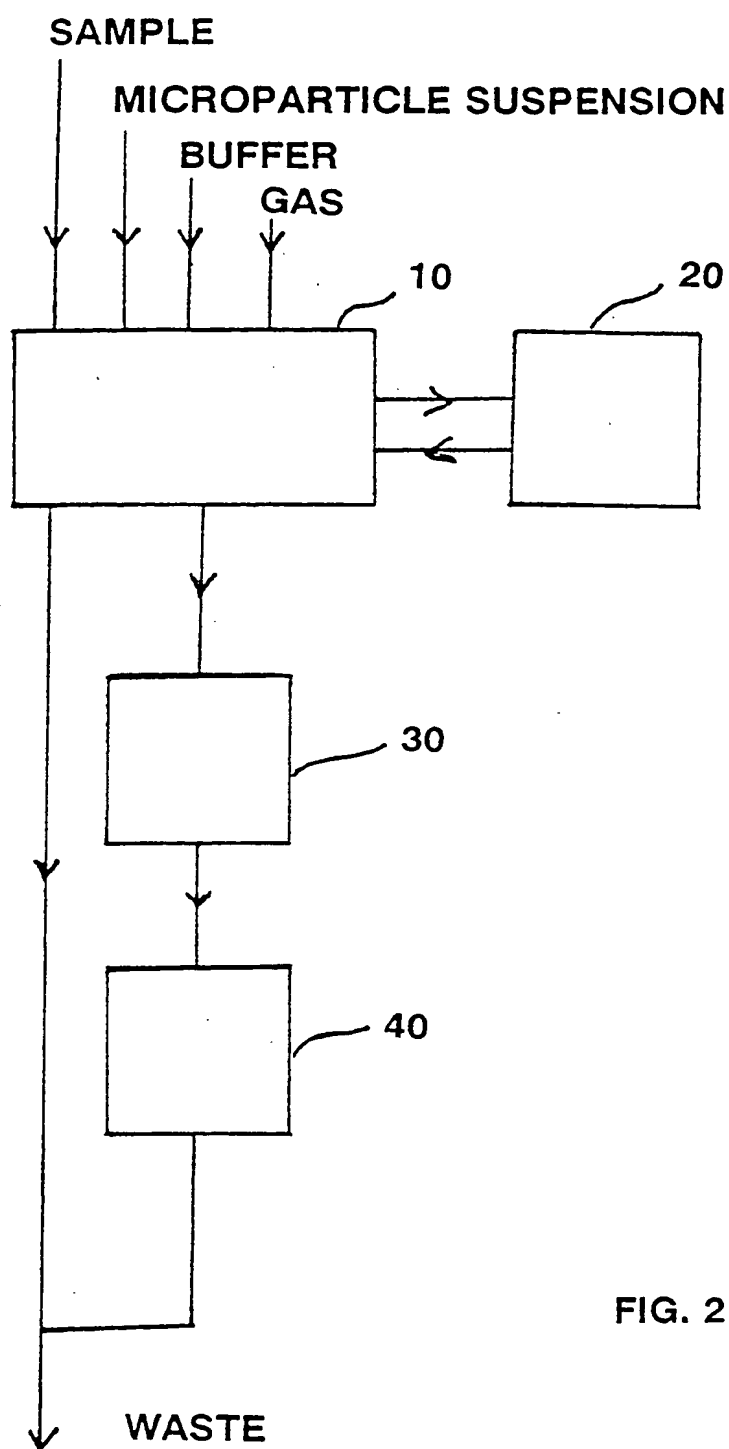


FIG. 2

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 93/00442

## A. CLASSIFICATION OF SUBJECT MATTER

IPC5: G01N 33/543, G01N 35/00 // G01N 21/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

INSPEC, WPI, MEDLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 5028545 (E J SOINI), 2 July 1991 (02.07.91), see, in particular column 6, line 21-24	1
Y	--	1-15
Y	FI, B, 86229 (N KAARTINEN), 15 April 1992 (15.04.92)	1-15
A	EP, A2, 0430248 (MOCHIDA PHARMACEUTICAL CO., LTD), 5 June 1991 (05.06.91)	1-15

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

\* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

14 February 1994

Date of mailing of the international search report

17 -02- 1994

Name and mailing address of the ISA/  
Swedish Patent Office  
Box 5055, S-102 42 STOCKHOLM  
Facsimile No. +46 8 666 02 86

Authorized officer

Carl Olof Gustafsson  
Telephone No. +46 8 782 25 00

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p data-bbox="342 216 1295 384">Dialog Information Services, File 154, Medline, Dialog accession no. 8676896, LJ Kricka et al: "Applications of a microfabricated device for evaluating sperm function", Clin Chem Sep 1993, 39 (9) p1944-7</p> <p data-bbox="695 405 829 457">-- -----</p>	1



## INTERNATIONAL SEARCH REPORT

Information on patent family members

28/01/94

International application No.

PCT/FI 93/00442

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US-A-	5028545	02/07/91	DE-A- 3870324 EP-A,B- 0296136	27/05/92 21/12/88
FI-B-	86229	15/04/92	NONE	
EP-A2-	0430248	05/06/91	AU-B- 642444 AU-A- 6702690 CA-A- 2031001 JP-A- 3223674 US-A- 5147607	21/10/93 06/06/91 31/05/91 02/10/91 15/09/92



## METHOD AND APPARATUS FOR BIOAFFINITY ASSAYS

This invention relates to a method and apparatus for performing and automating assay methods based on bioaffinity. The said method and apparatus are specifically used to perform and automate multiparameter assays based on  
5 the use of microparticles, especially in such as those described in U.S. patent No. 5,028,545 and the like.

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting  
10 the practice, are incorporated by reference.

Immunoassay is an assay method based on bioaffinity and widely used for biologically active molecules in clinical diagnostics and in research laboratories. Another important assay method based on bioaffinity is the DNA hybridization  
15 assay. The crucial reactant in bioaffinity assays is e.g. an antibody or a DNA probe, which is most often coupled to a solid support such as the wall of a test tube. The said reactant binds the molecule to be assayed (the analyte), and the specificity of the assay is determined above all by  
20 the characteristics of said reactant. Bioaffinity assays, which will be later also called biospecific assays, often involve a second antibody or a DNA probe labelled with, e.g., a fluorescent molecule. The complex generated in the biospecific reaction, which is bound to the solid phase,  
25 and comprises both biospecific reactants, the analyte molecule and the label, is separated from free labelled reactant, or the free fraction, by, e.g., washing the free fraction away, after which the strength of the signal produced by the label is measured by a suitable  
30 microphotometric method, which in case of fluorescent labels is a fluorometer, and in case of labels emitting chemiluminescence, a luminometer.

In diagnostics, the determination of a plurality of analytes in the same sample simultaneously may be required, this being denoted by the term "multiparameter assay". The multiparameter assays described in the literature so far  
5 are limited to the simultaneous assay of two to four analytes by using respectively two to four different labels whose signals allow sufficient spectrometric resolution.

In U.S. patent No. 5.028.545 a novel multiparameter assay method is described for the simultaneous determination of  
10 more than four analytes. This method makes use of microparticles (e.g., latex particles 10 to 200 micrometer) as the solid carrier, each separate particle forming a separate stoichiometric measuring basis in the respective reactant solution.

15 For each specified analyte microparticles are manufactured in separate batches such that different amounts of dye are added to the different batches (categories) of said particles for the purpose of identification of microparticle categories. The said dye may also be a  
20 fluorescent dye. The dye can be evenly distributed in the internal volume of the microparticle or on their surface. The reactant employed in a multiparameter assay method is a mixture of microparticles of different categories. The microparticles are, in addition, coated with the  
25 bioaffinity reactant for the specified analyte.

As described in U.S. patent No. 5,028,545, the biospecific binding reaction may be measured by means of a fluorescent label. The fluorescent label may be a lanthanide chelate with fluorescent excitation states of long lifetime (longer  
30 than 1 microsecond) compared to the lifetime of background fluorescence or the lifetime of the fluorescent excitation states of conventional organic dyes used to identifying microparticles. The fluorescence produced by lanthanide chelates is measured with a time-resolved fluorometer,  
35 which allows the efficient separation of the fluorescence

emitted by the lanthanide chelates from background fluorescence and from the fluorescence due to other organic dyes.

For the measurement of analyte concentrations by means of a  
5 fluorescent lanthanide chelate the time-resolved  
fluorometer can be used, in which the sample is excited  
with a short light pulse. For the measurement of the long-  
lived fluorescence of the lanthanide chelate and its  
separation from the background fluorescence of the  
10 microparticles, from scattering and from the signal emitted  
by the dye associated with each category, the light  
detector is only activated after a specified delay time  
following the excitation light pulse, and the short-lived  
fluorescence does not reach the light detector. The  
15 identification of the microparticle, on the other hand,  
takes place immediately during the excitation light pulse,  
or the system incorporates a separate photometric detector  
measuring light absorption or short-lived fluorescence.

The advantage of time-resolved fluorometry resides in the  
20 fact that the signal from the dye used for identification  
in no way interferes with the measurement of the signal  
from the lanthanide chelate, because the measurements are  
taken at different time points. This is why the system  
allows for the measurement of a plurality of analytes using  
25 microparticles in the same suspension.

An alternative label for detection of the bioaffinity  
reaction is the use of phosphorescent molecules. Certain  
metalloporphyrins, including platinum-coproporphyrin and  
palladium-coproporphyrin have been recently found to emit  
30 phosphorescence in water solution and introduced for use  
for labelling of biomolecules (A. P. Savitsky & al, Dokl.  
Acad. Nauk. USSR, 304, 1005, 1989). The time resolved  
measuring principle and the device needed for detection of  
the phosphorescence is essentially similar with the device  
35 normally used for detection of long decay time fluorescence

of lanthanide chelates.

Alternatively, a biospecific binding reaction may be measured also by means of a luminescent label, the said lanthanide chelate being replaced by a luminescent label.

- 5 The luminescent label may be in the form of a plurality of already known chemiluminescent or bioluminescent molecules. Examples of these are, among others, luciferase enzyme, derivatives of luminol, or the like. In this case microparticles are identified by a separate detector  
10 measuring light absorption or fluorescence, before the activation of the luminescent reaction. The dye used for identification does not interfere in any way with luminescence measurement, because the excitation and emission mechanisms are totally different and occur at  
15 different time points in the two cases.

Because each microparticle reacts on its own stoichiometrically, it is important that the particles be of exactly the same size. It is possible today to manufacture microparticles of precisely the same size for  
20 this purpose. If, however, the size distribution is too wide, an imaging light detector (e.g., a CCD element), may be used to identify the microparticles, by separate measurement of the diameter of each microparticle. The result is then utilized to perform the appropriate  
25 correction of the measurement data.

The aforementioned U.S. patent No. 5,028,545 does not describe the actual manner to carry out and automate this multiparameter assay based on bioaffinity. In general, fluid handling in the clinical and other analyzers ("auto-  
30 analysers") in use today is based on the use of test-tubes or microtiter plates, or fluids are handled in tubings controlled by peristaltic and piston pumps and various mechanical valves, in which tubings different samples are separated from each other by means of air bubbles, during  
35 the dispensing and transport of fluids. These apparatuses

are characterized by lack of provision for handling and transporting fluid volumes of less than 10 microliter or transporting microparticles one at a time at a specified speed. If in these apparatuses the internal diameter of the tubing is reduced to be almost equal to the external diameter of the microparticles, these microparticles may compromise the reliable operation of the mechanical valves. The fluid volumes handled in these apparatuses are generally at least tens of microliters, and cross-contamination is of the order of 0.1 to 1%. The concentration dynamics required in assays based on bioaffinity is at least 1000 times or, most often, 10000 times, and the use of conventional fluid handling elements and tubings in the field of application of this invention is made unacceptable by such cross-contamination levels.

Part of the fluid handling in conventional analyzers is also done in an open system. An open system in this connection denotes a system in which the samples or the reactants are, at some point of the assay, in open containers from which they may be dispensed or diluted or in which they may be incubated or measured. Examples of open analysis systems in general use are those in which samples are handled in test-tubes or microtiter plates. A closed analysis system in this connection denotes a system in which the samples and reactants are not contained in open vessels during the assay, nor are they in contact with air external to the system or with a gaseous phase in equilibrium with air. The problem with an open system is exposure of the samples and reactants to the chemical environmental factors and to evaporation as well as exposure of the personnel to possible pathogens in the samples. In addition, the surface tension of the fluids restricts the possibility to dispense microvolumes of 1 microliter or less, and in practice the sample volumes in all open systems during the process are more than 10 microliter or even more than 100 microliter.

The multiparameter assay method based on bioaffinity and the use of microparticles, described in U.S. patent No. 5,028,545, cannot be automated by applying the aforementioned conventional principles, because open fluid handling systems do not allow for precise transfer or dispensing of single or a small number of microparticles contained in volumes smaller than 1 microliter. The object of this invention is to describe the manner in which to construe an automated multiparameter bioaffinity micro-assay system comprising microparticles as a solid reaction support and a system of microchannels for fluid handling. A system of microchannels in this connection denotes channels wider by, e.g., twice the external dimensions of the microparticles used for this purpose, the said channels being in the form of either thin tubes or thin channels manufactured inside a solid support by etching or engraving or by some other suitable method, the said channels connecting separate fluid handling operations, and along which channels suspensions of microparticles are transferred from one point to another. The microchannels have a geometric design such as allows the transfer and handling of microparticles in the form of a fluid suspension while avoiding obstruction of the system of channels by the particles or their remaining in pockets of the system of channels. The internal dimensions of the system of microchannels are selected such as to allow movement of microparticles in said system of microchannels, centered by laminar flow, in the center of the microchannel, singly in a row. Those parts of the system of microchannels which are intended for storing samples or for incubation, e.g., the sample register, may, however, be of wider internal dimensions. If rather large microparticles, e.g., larger than 100 micrometer, are used, a suitable internal diameter is 250 micrometer for a microchannel intended for the transfer of a suspension of microparticles. If smaller microparticles, e.g., of the size of 10 micrometer, are used, a suitable internal diameter is e.g. 50 micrometer for a microchannel intended



for the transfer of a suspension of microparticles.

The purpose of the use of a system of microchannels is to use microvolumes and at the same time make savings in the expenditure for reagents. Cost savings are realized by  
5 reducing the amounts of specific reactants to a fraction, e.g., a hundredth of the volumes used in modern open systems. In a closed analysis system designed according to this invention, based on a system of microchannels, samples of a volume less than 1 microliter or even less than 100  
10 nanoliter may be handled quantitatively with good precision. In addition, this invention allows a plurality of simultaneous different bioaffinity determinations, that is, a specified diagnostic panel, or the combination of all the analytes relevant to specified diagnostic symptoms, for  
15 instance, to be carried out in the said volume. In practice a capacity of ten analytes is probably sufficient, although the said system probably allows an even greater number of analytes to be measured simultaneously. The adoption of the said microassay system possibly allows routine diagnostics  
20 to be simplified and the extent of manual handling and the amounts of reactants and packaging material to be reduced. In reagent production the said system will also allow great economies. The production of diagnostic test kits for open systems involves bulky fluids, coated test-tubes and their  
25 packaging and also requires a high standard of hygiene. The production system for reactants for a closed microassay system such as described here may be construed as a closed process.

The object of this invention is to describe the manner in  
30 which an assay method of the aforesaid characteristics can be completely automated by applying the concept of a system of microchannels. Microparticles have been observed to move in the microchannels, centered by laminar flow, onto the center axis of the channel, one after the other, in a  
35 controlled manner. All stages of handling of microparticles are carried out in a system of microchannels according to

this invention, including at least the identification of categories and measurement, and according to the capacity of the apparatus, possibly also dispensing, mixing, incubation, and the separation of the free fraction. A  
5 sample register is formed by the microchannels and valves to allow simultaneous incubation of a plurality of samples.

The characteristic features of the invention are stated in claim 1.

10 Mixing of samples and reactants in connection with dispensing and during incubation is effected by turbulent flow generated by hydraulic pressure pulses in the microchannel, which contains, in addition to the  
aforementioned fluids, a gas phase which provides an  
15 elastic cushion to receive the pressure pulses. Mixing is effected in such a manner that the fluids are not in contact with moving wear-sensitive parts. The free and the bound labelled reactant are separated by exchanging the liquid phase in the microchannel containing the  
20 microparticles.

One system of microchannels satisfying the aforesaid requirements is the system of "microfluidistics" whose operational principles and components have been described in, among others, the following Finnish patents: Nos.  
25 56591, 57849, 57850, 64012, 67490, 70331, 71102, 72660, and 86229. After the publication of the aforementioned patents more sample handling methods, components and their production methods based on microfluidistics have been developed, such as a dispensing needle for the sterile  
30 recovery of the sample from the primary sample tube, and a method for the manufacture of the said needle, a method for the mixing of a fluid volume in a vessel during dispensing by means of the dispensing needle, and a nonlinear dispenser based on a metal bellows, the accuracy of the  
35 dispenser being constant at all volumes.

The microfluidistics system described in the aforementioned patents allows precise handling and transport of small reactant volumes (less than 1 microliter) and small numbers of microparticles or even of single particles, in a closed  
5 system of microchannels. The said system is a hermetically sealed, sterile if need be, quantitative system for fluid handling, composed of thin-walled microchannels of inert metal. Fluid handling is directed through electrically controlled ice valves, in which valves the fluid (sample,  
10 reactant or auxiliary fluid) is frozen or defrosted to close or to open the parts functioning as gates for the microchannels. The volume of the valves is only, e.g., 50 nanoliters, which means that they have a reduced heat capacity and consequently their operation is fast. An  
15 important advantage of the said ice valves in a system designed according to this invention is provided by the fact that the microparticles do not interfere mechanically with the functioning of the ice valves, even though their external dimensions were almost equal to the internal  
20 dimensions of the microchannel.

An alternative technology of manufacturing microfluidistics is the use of crystalline silicon as solid substrate. The microchannels and other necessary structures can be etched  
25 in this silicon using a planar photolithographic micromachining techniques developed by microelectronics industry (M. Salonen and T. Ropponen, Mikromekaniikka, Helsinki University of Technology, Publication no. C234, 1991). Standard silicon wafers with a suitable crystalline  
30 orientation and diameter e.g. 100 mm or larger can be used. The connections can be etched into the silicon wafer on either side and the liquid is supplied or removed from the microchannel system through vertical bores.

The upper face of the silicon wafer consists of thin quartz  
35 glass which is hermetically attached to silicon using standard heating technique. The quartz cover permits direct

flow observation and detection of photon emission from the luminescent, fluorescent or phosphorescent labels of the reactants on the microparticles. Silicon wafers used by semiconductor industry is chemically extremely pure and according to our test results does not contain any impurities, e.g. heavy metals which would interfere with the fluorescence detection of the lanthanide chelates. Certain types of quartz also fulfill the purity requirements.

- 10 Microchannels for liquid handling can be etched in silicon with precision of 0.1 micrometers (J. Phaler & al., Liquid transport in micron and submicron channels, Sensors and Actuators, A21 A23 (1990) 431-434). This precision permits constructions of reaction chambers where the microparticles are kept inside a geometrically precisely defined part of a microchannel whose wall is a filter. The principal construction of a such reaction chamber is shown in Figure 1. This construction allows washing of the free fraction of the labelled reactants without moving the microparticles.
- 15 The microparticles are fed in through the input 1. and fed out through the output 2. The washing is performed simply by a flow of washing solution through the filter from the input 3 and the output 4. The filter 5 prevents the microparticles 6 to move in the direction of the wash flow.
- 20 The filter consists of parts of the silicon having any optimal form and dimensions. It is obvious that the spacing between the parts shall be smaller than the diameter of the microparticle.

- Most of the microfluidistics functions described in the patents referred above, can be performed also by the constructions in silicon, including for example ice valves. Silicon technology allows to integrate silicon with metal constructions and metals with high thermal conductivity can be used as "cold fingers" for operating the ice valves.
- 30 High thermal insulation between the cold fingers and other construction can be achieved with built-in vacuum cavities.